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APPLICATION NUMBER: *60/487,553*
FILING DATE: *July 14, 2003*
RELATED PCT APPLICATION NUMBER: *PCT/US04/22850*

Certified by

Jon W Dudas

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Donkena Krishna	**Vanaja**	**Rochester, Minnesota**

☒ *Additional inventors are being named on the 1 separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

METHODS AND COMPOSITIONS FOR DIAGNOSIS, STAGING AND PROGNOSIS OF PROSTATE CANCER

CORRESPONDENCE ADDRESS

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ENCLOSED APPLICATION PARTS ***(check all that apply)***

☒ Specification *Number of Pages* **19** ☐ CD(s), Number

☒ Drawing(s) *Number of Sheets* **7** ☒ Other (specify) **Fee Transmittal (+ copy).**

☐ Application Data Sheet. See 37 CFR 1.76 **Sequence Listing (printed only), Check**

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☒ Applicant claims small entity status. See 37 CFR 1.27.

☒ A check or money order for $*80* is enclosed to cover the filing fees.

☐ The Commissioner is hereby authorized to charge filing fees to Deposit Account Number: **04-0258**

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☐ No.

☒ Yes, the name of the U.S. Government agency and the Government contract number are: **NIH CA91956 and CA70892**.

Respectfully submitted,			
SIGNATURE		DATE	**July 14, 2003**
TYPED or PRINTED NAME	**Barry L. Davison**	REGISTRATION NO. *(if appropriate)*	**47,309**
TELEPHONE	**206-628-7621**	DOCKET NUMBER:	**88-88**

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

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TOTAL AMOUNT OF PAYMENT ($) 80

Complete if Known	
Application Number	To be assigned
Filing Date	July 14, 2003
First Named Inventor	Vanaja
Examiner Name	
Art Unit	
Attorney Docket No.	88-88

METHOD OF PAYMENT *(check all that apply)*

☒ Check ☐ Credit card ☐ Money Order ☐ None

☒ Deposit Account:

Deposit Account Number: 04-0258

Deposit Account Name: Davis Wright Tremaine LLP

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FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee($)	Small Entity Fee Code	Small Entity Fee($)	Fee Description	Fee Paid
1001	750	2001	375	Utility filing fee	
1002	330	2002	165	Design filing fee	
1003	520	2003	260	Plant filing fee	
1004	750	2004	375	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	80
				SUBTOTAL (1)	($)80

2. EXTRA CLAIM FEES

			Extra Claims		Fee from below	=	Fee Paid
Total Claims		- 20** =		x		=	
Independent Claims		- 3** =		x		=	
Multiple Dependent						=	

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	84	2201	42	Independent claims in excess of 3
1203	280	2203	140	Multiple dependent claim, if not paid
1204	84	2204	42	** *Reissue independent claims over original patent*
1205	18	2205	9	** *Reissue claims in excess of 20 and over original patent*

SUBTOTAL (2) ($)0

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FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet.	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	410	2252	205	Extension for reply within second month	
1253	930	2253	465	Extension for reply within third month	
1254	1,450	2254	725	Extension for reply within fourth month	
1255	1,970	2255	985	Extension for reply within fifth month	
1401	320	2401	160	Notice of Appeal	
1402	320	2402	160	Filing a brief in support of an appeal	
1403	280	2403	140	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive – unavoidable	
1453	1,300	2453	650	Petition to revive – unintentional	
1501	1,300	2501	650	Utility issue fee (or reissue)	
1502	470	2502	235	Design issue fee	
1503	630	2503	315	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Petitions related to provisional applications	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	750	2809	375	Filing a submission after final rejection (37 CFR § 1.129(a))	
1810	750	2810	375	For each additional invention to be examined (37 CFR § 1.129(b))	
1801	750	2801	375	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	
Other fee (specify) ____					

*Reduced by Basic Filing Fee Paid SUBTOTAL (3) ($)0

SUBMITTED BY

Name (Print/Type) Barry L. Davison	Registration No. Attorney/Agent) 47,309	22504 PATENT TRADEMARK OFFICE
Signature	Date July 14, 2003	

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PROVISIONAL APPLICATION COVER SHEET
Additional Page

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	88-88	Type a plus sign (+) inside this box →	+

INVENTOR(S)/APPLICANT(S)

Given Name (first and middle [if any])	Family or Surname	Residence (City and either State or Foreign Country)
Charles Y.F.	Young	Rochester, Minnesota

Number 2 of 2

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EXPRESS MAIL NO.: EV 284452731 US
DOCKET NO.:

METHODS AND COMPOSITIONS FOR DIAGNOSIS, STAGING AND PROGNOSIS OF PROSTATE CANCER

FIELD OF THE INVENTION

The present invention relates, *inter alia*, to novel methods and compositions for the diagnosis, staging and prognosis of prostate cancer, based on transcriptional silencing of gene expression, including of zinc finger protein 185 (ZNF 185), prostate secretory protein (PSP94) and bullous pemphigoid antigen (BPAG). The present invention also relates to genomic DNA methylation.

STATEMENT REGARDING FEDERALLY FUNDED RESEARCH

This work was, at least in part, supported by National Institutes of Health Grants CA91956 and CA70892, and the United States Government has certain rights in the invention.

BACKGROUND

Currently, tumor stage, Gleason score, and preoperative serum PSA are the only well-recognized predictors of prostate cancer progression. However, these markers cannot reliably identify men that ultimately fail therapy, and give no insight into prostate carcinogenesis, or potential therapeutic targets for prostate cancer.

Inactivation of tumor suppression genes is an important event contributing to the development of neoplastic malignancies. In addition to the classical genetic mechanisms involving deletion or activating point mutations, growth regulatory genes can be functionally inactivated by epigenetic alterations, for example, alterations in the genome other than the DNA sequence itself; which include global genomic hypomethylations, promoter hypermethylation of CpG islands, histone deacetylations and chromatin modifications. Molecular analysis of tumor-derived genetic and epigenetic alterations may have a profound impact on cancer diagnosis and monitoring for tumor recurrence.

There is a need in the art to analyze differentially expressed genes (*e.g.*, using microarrays) between corresponding normal and cancer tissues to advance the understanding of the molecular basis of malignancy, and provide biomarkers or prognostic markers of malignancy. There is a need

in the art to identify and statistically correlate altered expression of genes that is characteristic of the specific stage of the cancer to provide a supplementary approach to the histopathological work-up of precancerous and cancerous lesions of the prostate.

SUMMARY OF THE INVENTION

Genes expression was profiled in benign and untreated human prostate cancer tissues using oligonucleotide microarrays. Fifty (50) genes with distinct expression patterns in metastatic and confined tumors (Gleason score 6 and 9, lymph node invasive and non-invasive) were identified. Validation of expression profiles of six genes by quantitative PCR revealed a strong inverse correlation in the expression of zinc finger protein (ZNF185), bullous pemphigoid antigen gene (BPAG1), and prostate secretory protein (PSP94) with progression of prostate cancer.

Treatment of prostate cancer cell lines with 5-aza-2'-deoxycytidine, an inhibitor of DNA methylation, restored ZNF185 expression levels. Moreover, methylation-specific PCR confirmed methylation of the 5'CpG islands of the ZNF185 gene in all metastatic tissues and 44% of the localized tumor tissues as well as in the prostate cancer cell lines tested. Thus, transcriptional silencing of ZNF185 by DNA methylation in prostate tumor tissues implicates the ZNF185 gene in prostate tumorigenesis.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows expression of 50 significantly regulated genes in 36 prostate tissue samples. Cluster diagram depicting genes that distinguish metastatic (Met; n=5) from confined tumors with Gleason score 9 lymph node positive (9P; n=6) or negative (9N; n=6) and Gleason score 6 lymph node positive (6P; n=6) or negative (6N; n=5) prostate cancer and adjacent benign tissues (ABT; n=8) (n represents the number of tissues). Each row represents a gene and each column a tissue sample. Red and green represent up regulation and down regulation, respectively, relative to the median of the reference pool. Gray represents technically inadequate or missing date, and black represents equal expression relative to the reference samples. Color saturation is proportional to the magnitude of the difference from the mean. Each gene is labeled by its gene name. Mean and standard deviation (S.D.) of the fold change in the expression levels of genes compared to ABT is shown.

Figure 2a shows forward primer (FP), reverse primer (RP) and probes used for Taqman real-time PCR.

Figure 2b shows expression levels of genes ZNF185, PSP94, BPAG1 and Erg-2 as validated by Taqman real-time PCR in 36 samples (28 cancer and 8 benign) used for microarray analysis and an additional 8 samples (4 cancer and 4 benign). Values are expressed as the copy number of the gene relative to GAPDH levels. Metastatic tissues (Met υ) n=5, Gleason score 9, lymph node positive (9P ■) n=7 or negative (9N □) n=8 and Gleason score 6, lymph node positive (6P λ) n=6 or negative tissues (6N ○) n=6 and adjacent benign tissues (ABT σ) n=12 were used. (n represents the number of tissues). Mean ± standard deviation (S.D.) of relative expression levels of each group is shown on the left.

Figure 3a shows expression of ZNF185 levels in prostate cancer cells treated with 6 μM 5-Aza-CdR for 6 days. Four separate experiments are represented, and the error bars denote the standard deviation. The symbol "*" Indicates statistical significance over the untreated cells (p<0.05%).

Figure 3b shows the PCR primers (forward primer [FP], reverse primer [RP]), used for MSP of prostate tissues. The symbol "W" represents unmodified or wild type primers, "M," methylated-specific primers, and "U," unmethylated-specific primers. equence difference between modified primers and unmodified DNA are in boldface type and differences between methylated/modified and unmethylated/modified are underlined.

Figure 3c shows MSP analysis of ZNF185 DNA in prostate tissue samples and cell lines, with and without 5-Aza-CdR treatment. The amplified products were directly loaded onto DNA 500 lab chip and analyzed on Agilent 2100 Bioanalyzer. Molecular size marker is shown at left. All DNA samples were bisulfite-treated except those designated untreated. The experiments were repeated twice and the representative band of the PCR product in lanes U, M and W indicates the presence of unmethylated, methylated and wild type ZNF185 DNA, respectively.

Figure 3d shows a summary of the incidence of methylation of ZNF185 DNA in prostate tissues analyzed by MSP.

DETAILED DESCRIPTION OF THE INVENTION

DEFINITIONS

The term "ZNF185" refers to the art recognized zinc finger protein 185;

The term "PSP94" refers to Prostate secretory protein 94;

The term "BPAG1" refers to Bullous pemphigoid antigen gene 1; and

The term "MSP" refers to Methylation specific PCR

The terms "LNCaP," "PC3" and "LAPC4" refer to art-recognized human prostate cancer cell lines.

OVERVIEW

The present invention provides, *inter alia*, biologically and clinical relevant clusters of genes characteristic of prostate cancer versus benign tissues and confined versus metastatic prostate cancer using oligonucleotide microarrays. Expression profiles were generated from 5 metastatic prostate tissues, and 23 confined tumors including 12 Gleason score 9 (high grade), and 11 Gleason score 6 (intermediate grade) tumors. In addition, 8 adjacent benign prostatic tissues were also studied. Fifty (50) genes have been identified herein with distinct expression patterns in prostate cancer compared with benign prostatic tissues. Expression levels of prostate secretory protein (PSP94), zinc finger protein (ZNF185), bullous pemphigoid antigen gene (BPAG1), prostate specific transglutaminase gene (TGM4), Erg isoform 2 (Erg-2) and Rho GDP dissociation inhibitor (RhoGD-β) were validated by Taqman quantitative real-time PCR. Furthermore, analysis of the expression of ZNF185 in prostate cancer cell lines revealed an increase in the expression by treatment with an inhibitor of DNA methylation, 5-aza-2'-deoxycytidine. Methylation specific PCR (MSP) indicated ZNF185 inactivation by CpG dinucleotide methylations in prostate cancer cell lines and cancer tissues. Our studies show that down-regulation of ZNF185, PSP94 and BPAG1 with epigenetic alteration of ZNF185 is highly associated with prostate cancer progression and serve as useful biomarkers for predicting progression of the cancer.

Oligonucleotides. The present invention includes nucleic acid molecules (*e.g.*, oligonucleotides and peptide nucleic acid (PNA) molecules (PNA-oligomers)) that hybridize under moderately stringent and/or stringent hybridization conditions to all or a portion of the sequences

SEQ ID NO:1, or to the complement thereof. The hybridizing portion of the hybridizing nucleic acids is typically at least 9, 15, 20, 25, 30 or 35 nucleotides in length. However, longer molecules have inventive utility, and are thus within the scope of the present invention.

Preferably, the hybridizing portion of the inventive hybridizing nucleic acids is at least 95%, or at least 98%, or 100% identical to the sequence, or to a portion thereof of SEQ ID NO:1, or to the complement thereof.

Hybridizing nucleic acids of the type described herein can be used, for example, as a primer (*e.g.*, a PCR primer), or a diagnostic and/or prognostic probe or primer. Preferably, hybridization of the oligonucleotide probe to a nucleic acid sample is performed under stringent conditions and the probe is 100% identical to the target sequence. Nucleic acid duplex or hybrid stability is expressed as the melting temperature or Tm, which is the temperature at which a probe dissociates from a target DNA. This melting temperature is used to define the required stringency conditions.

For target sequences that are related and substantially identical to the corresponding sequence of SEQ ID NO:1 (such as allelic variants and SNPs), rather than identical, it is useful to first establish the lowest temperature at which only homologous hybridization occurs with a particular concentration of salt (*e.g.*, SSC or SSPE). Then, assuming that 1% mismatching results in a 1°C decrease in the Tm, the temperature of the final wash in the hybridization reaction is reduced accordingly (for example, if sequences having > 95% identity with the probe are sought, the final wash temperature is decreased by 5°C). In practice, the change in Tm can be between 0.5°C and 1.5°C per 1% mismatch.

Examples of inventive oligonucleotides of length X (in nucleotides), as indicated by polynucleotide positions with reference to SEQ ID NO:1, include those corresponding to sets (sense and antisense sets) of consecutively overlapping oligonucleotides of length X, where the oligonucleotides within each consecutively overlapping set (corresponding to a given X value) are defined as the finite set of Z oligonucleotides from nucleotide positions:

n to (n + (X-1));

where n=1, 2, 3,...(Y-(X-1));

where Y equals the length (nucleotides or base pairs) of SEQ ID NO:1 (3,614);

where X equals the common length (in nucleotides) of each oligonucleotide in the set (*e.g.*, X=20 for a set of consecutively overlapping 20-mers); and

where the number (Z) of consecutively overlapping oligomers of length X for a given SEQ ID NO of length Y is equal to Y-(X-1). For example Z= 3,614-19= 3,595 for either sense or antisense sets of SEQ ID NO:1, where X=20.

Preferably, the set is limited to those oligomers that comprise at least one CpG, TpG or CpA dinucleotide.

Examples of inventive 20-mer oligonucleotides include the following set of 3,595 oligomers (and the antisense set complementary thereto), indicated by polynucleotide positions with reference to SEQ ID NO:1:

1-20, 2-21, 3-22, 4-23, 5-24, ...3593-3612, 3594-3613 and 3595-3614.

Preferably, the set is limited to those oligomers that comprise at least one CpG, TpG or CpA dinucleotide.

The present invention encompasses, for SEQ ID NO:1 (sense and antisense), multiple consecutively overlapping sets of oligonucleotides or modified oligonucleotides of length X, where, *e.g.*, X= 9, 10, 17, 20, 22, 23, 25, 27, 30 or 35 nucleotides.

The oligonucleotides or oligomers according to the present invention constitute effective tools useful to ascertain genetic and epigenetic parameters of the genomic sequence corresponding to SEQ ID NO:1, and the complement thereof. Preferred sets of such oligonucleotides or modified oligonucleotides of length X are those consecutively overlapping sets of oligomers corresponding to SEQ ID NO:1 (and to the complement thereof). Preferably, said oligomers comprise at least one CpG, TpG or CpA dinucleotide.

Oligonucleotides and PNA-oligomers capable of hybridizing, as described herein above, to bisulfite-converted sequences of SEQ ID NO:1 are also within the scope of the present invention.

EXAMPLE 1

(A set of genes was identified that characterize prostate cancer and benign prostatic tissues)

Materials and methods

Prostate tissues. Prostate cancer tissue specimens were obtained from patients who had

undergone radical prostatectomy for prostate cancer at Mayo Clinic. The Institutional Review Board of Mayo Foundation approved collection of tissues, and their use for this study. None of the patients included in this study had received preoperative hormonal therapy, chemotherapy, or radiotherapy. Harvested tissues were embedded in OCT and frozen at –80°C until use. A hematoxylin and eosin stained section was prepared to insure that tumor was present in the tissue used for the analyses. Out of 340 tissues available in our tissue bank, we selected tissues that had more than 80% of the neoplastic cells by histological examination. In order to examine differential gene expression in intermediate (Gleason score 6), high grade (Gleason score 9) prostatic adenocarcinoma and metastatic tumors, we studied 11 primary stage T2 Gleason score 6 cancers (six with positive regional lymph nodes and five with negative lymph nodes), 12 primary stage T3 Gleason score 9 cancers (six with positive regional lymph nodes, six with negative lymph nodes), and five metastatic tumors.

TABLE 1 shows Gleason grade, age, pre-operative serum prostate-specific antigen levels and staging of all patients from whom prostate tissues were obtained for this study. Twelve separately collected prostatic tissue samples matched with the cancer tissues (obtained from the same patients) were used as normal controls.

Isolation of RNA and gene expression profiling. Thirty prostate tissue sections of 15-μm thicknesses were cut with a cryostat and used for RNA isolation. Total RNA was extracted from frozen tissue sections with Trizol® reagent (Life Technologies, Inc., Carlsbad, CA). DNA was removed by treatment of the samples with DNase I using DNA-free™ kit (Ambion, Austin, TX) and further RNA cleanup was performed using RNeasy Mini kit (Qiagen, Valencia, CA) according to the manufacturer's protocols. RNA quality was monitored by agarose gel electrophoresis and also on Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). High-density oligonucleotide microarrays HG-U95Av2 containing 12,625 sequences of human genes and ESTs (Affymetrix, Santa Clara, CA) were used in this study. Complementary RNA was prepared, labeled and hybridized to oligonucleotide arrays as described previously (13). The arrays were scanned with gene array scanner (Agilent technologies, Palo Alto, CA). All arrays were scaled to a target intensity of 1500. Raw data was collected and analyzed by using Affymetrix Microarray Suite 5.0 version.

Quantitative Real-Time RT-PCR. To confirm the differential expression of genes from

microarray data, four down-regulated genes, ZNF185, PSP94, BPAG1 and TGM4 and two up-regulated genes Erg-2 and RhoGDI-β were selected for validation by Taqman real-time RT-PCR in a total of 44 tissues, including 36 samples used for microarrays with an additional 4 primary tumors and 4 adjacent benign tissues. One (1) μg of the total RNA was used for first-strand cDNA synthesis. The PCR mix contained 1X reaction buffer (10 mM Tris, 50 mM KCl, pH 8.3), $MgCl_2$ (5 mM), PCR nucleotide mix (1 mM), random primers (0.08 A260 units), RNase inhibitor (50 units), AMV reverse transcriptase (20 units) in a final volume of 20 μl.

For real-time PCR one μl of the cDNA was used in the PCR reactions. Taqman real-time primers and probes were designed using the software Primer Express™ version 1.5 (PE Applied Biosystems, Foster City, CA) and synthesized at Integrated DNA Technologies (Coralville, IA). The sequences of the primers and probes for each gene are provided in Fig. 2(a). Probes were labeled at 5' end with the reporter dye 6-carboxyfluorescein (6'-FAM) and at 3' end with a Black Hole Quencher (BHQ). Probes were purified by reverse phase HPLC and primers were PAGE purified. All PCR reactions were carried out in Taqman Universal PCR master mix (PE Applied Biosytems) with 300 nM of each primer and 200 nM of probe in a final volume of 50 μl. Thermal cycling conditions were as follows: 2 min at 50°C, with denaturation at 95°C for 10 min, 40 cycles of 15 sec at 95°C (melting) and 1 min at 60°C (annealing and elongation). The reactions were performed in an ABI Prism® 7700 Sequence Detection System (PE Applied Biosystems). To evaluate the validity and sensitivity of real-time quantitative PCR, serial dilutions of the oligonucleotide amplicon of the gene in a range of 1 to 1 x 10^9 copies were used as corresponding standard. Standard curves were generated using the C_t values determined in the real-time PCR to permit gene quantification using the supplied software according to the manufacturer's instructions. In addition, a standard curve was generated for the housekeeping gene, glyceraldehyde-3-phosphate-dehydrogenase (Applied Biosystems, part number 402869) to enable normalization of each gene. Data were expressed as relative copy number of transcripts after normalization.

Cell Lines and 5-Aza-CdR Treatment. The human prostate cancer cell lines LNCaP, PC3 (American Type Culture Collection, Rockville, MD, USA) and LAPC4 (a gift from Dr. Charles L. Sawyers, University of California, Los Angeles, CA) were grown in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 5% fetal bovine serum (FBS) at 37°C and 5%

CO_2 until reaching approximately 50-70% confluence. Cells were then treated with 5% FBS RPMI 1640 containing 6μM 5-aza-2'-deoxycytidine (5-Aza-CdR) (Sigma Chemicals Co., St. Louis, MO) for 6 days, with medium changes on day 1, 3, and 5. Total RNA was isolated from the cell lines and the expression of the ZNF185 was analyzed by Taqman real-time PCR as described above. The housekeeping gene GAPDH was used as an internal control to enable normalization.

DNA isolation and Bisulfite modification. Genomic DNA was obtained from metastatic, primary, matched benign prostatic tissues and the above mentioned prostate cancer cell lines treated with 5-Aza-CdR, using Wizard® genomic DNA purification kit according to the manufacturer's protocol (Promega, Madison, WI). Genomic DNA (100 ng) was modified by sodium bisulfite treatment by converting unmethylated, but not methylated, cytosines to uracil as described previously (14). DNA samples were then purified using the spin columns (Qiagen), and eluted in 50 μl of distilled water. Modif ication was completed by treatment with NaOH (0.3 M final concentration) for 5 min at room temperature, followed by ethanol precipitation. DNA was re-suspended in water and used for PCR amplification.

Methylation Specific PCR (MSP). DNA methylation patterns within the gene were determined by chemical modification of unmethylated cytosine to uracil and subsequent PCR as described previously (15), using primers specific for either methylated or the modified unmethylated sequences. The primers used for MSP were shown in Fig. 3(b). Two sets of primers were designed corresponding to the genomic positions around 210 and 335. Genomic position indicates the location of the 5' nucleotide of the sense primer in relation to the major transcriptional start site defined in the Genbank accession number (Y09538). The PCR mixture contained 1X PCR buffer (50 mM KCl, 10 mM Tris-HCl pH 8.3 with 0.01% w/v gelatin), dNTPs (0.2 mM each), primers (500 μM) and bisulfite modified or unmodified DNA (100 ng) in a final volume of25 μl. Reactions were hot-started at 95°C for 10 min with the addition of 1.25 units of AmpliTaq Gold ™ DNA polymerase (PerkinElmer). Amplifications were carried out in GeneAmp PCR systems 9700 (Applied Biosystems) for 35 cycles (30 sec at 95°C, 30 sec at 55°C and 30 sec at 72°C), followed by a final 7 min extension at 72°C. Appropriate negative and positive controls were included in each PCR reaction. One (1) μl of the PCR product was directly loaded onto DNA 500 lab chip and analyzed on Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto,CA).

RESULTS

Gene expression profiles of 28 prostate cancer tissues were monitored using oligonucleotide microarrays. A gene-by-gene analysis of the difference in mean log expression between the two groups was performed to identify genes differentially expressed between cancer and benign tissues. Genes were ranked according to inter-sample variability (SD), and 1850 genes with the most variable expression across all of the samples were median-centered and normalized with respect to other genes in the samples and corresponding genes in the other samples. Genes and samples were subjected to hierarchical clustering essentially as described previously (16). Differential expression of genes in benign and malignant prostate tissues was estimated using an algorithm (13) based on equally weighted contributions from the difference of hybridization intensities (μTumor-μNormal) or (μNormal-μTumor), the quotient of hybridization intensities (μTumor/μNormal) or (μNormal/μTumor), and the result of an unpaired *t*-test between expression levels in tumor and normal tissues. The selection criteria was narrowed to genes that showed a fold change of >2.35 between normal and cancer samples and a $p<0.001$ by student's t-test. A cluster of 25 up-regulated and 25 down-regulated genes, which discriminated between normal and cancer tissues was identified (Fig. 1).

Among the 25 down-regulated genes we identified (Fig.1), PSP94, BPAG1, WFDC2, KRT5, KRT15, TAGLN, ZFP 36 and the genes encoding LIM domain proteins FLH1, FLH2, ENIGMA are consistent with the expression profiles of the previous studies (2, 7, 17-19). Up-regulation of hepsin, AMACR, STEAP, FOLH1, RAP2A and the unknown gene DKFZP564B167 are consistent with the previously published data of microarray analysis (2-7, 18, 20, 21). In addition our data also confirms up-regulation of the cell cycle regulated genes CCNB1, CCNB2, MAD2L1, DEEPEST, BUB1B, cell adhesion regulator MACMARCKS, unclassified genes KIAA0186 and KIAA0906 (5,7, 17, 21).

PSP94, ZNF185, BPAG1, and TGM4 were selected from the 25 down-regulated genes and Erg-2 and RhoGDI-β from the 25 up-regulated genes for further validation by Taqman quantitative PCR. These genes were selected because of their moderate to high level expression in prostate cancer. In addition, their potential functions, as mentioned below, are relevant to prostate cancer

biology. Furthermore, except for PSP94, their role in prostate cancer biology has not been previously described. PSP94 has been shown to be down-regulated in prostate cancer (22) and is the most down-regulated gene in the instant microarray data.

To validate the expression profiles, Taqman quantitative PCR was performed in duplicate for each sample. The standard curve slope values for all the genes ranged between –3.58 and –3.20, corresponding to PCR efficiency of above 0.9. The Kruskal-Wallis global test was done with the real time quantitative analysis for all the genes. A significant decrease in the expression of ZNF185, BPAG1 and PSP94 mRNA levels was observed in metastatic *versus* organ confined and localized tumors compared to benign tissues [$p<0.0001$] (Fig. 2b). Moreover, the Wilcoxon test was used to compare each tissue type to the adjacent benign tissues. ZNF185, BPAG1 and PSP94 showed p-values less than 0.0019 in each group compared to benign tissues.

PSP94 is a highly prostate specific gene encoding a major prostate secretory protein. Earlier studies reported that both the secretion and synthesis of PSP94 were reduced in prostate cancer tissues (22). PSP94 is involved in inhibition of tumor growth by apoptosis (23) and the down-regulation in prostate tumor tissues may be the survival mechanism for cancer cells. The instant experiments indicate that PSP94 palys a role in prostate cancer progression.

BPAG1 is a 230-kDa hemi-desmosomal component involved in adherence of epithelial cells to the basement membrane. Previous studies have shown a loss of BPAG1 in invasive breast cancer cells (24). The down-regulation of BPAG1 in our study (>14 fold in metastatic tissues) provides an indicator of an invasive phenotype and predicts the potential of invasive cells to metastasize (25).

Erg-2 is a proto-oncogene known to play an important role in the development of cancer (26). Erg-2 expression levels were herein observed to increased in 16 (50%) out of 32 cancer tissues when stringently compared to the highest level of Erg-2 in 12 adjacent benign tissues. The increase in mRNA levels of Erg-2 in at least half of the cancer tissues examined indicates a role of Erg-2 in prostate cancer.

Furthermore, TGM4 is a prostate tissue specific transglutaminase (type IV) that has been implicated in apoptosis and cell growth (27). RhoGDI- β may be involved in cellular transformation (28). The present Taqman PCR study shows that TGM4 and RhoGDI- β levels were not changed significantly in most of the prostate cancer tissues (data not shown).

ZNF185 is a novel LIM domain gene (29), and, according to the present invention, plays a role in prostate cancer development and progression. Particular LIM domain proteins have been shown to play an important role in regulation of cellular proliferation and differentiation (30-34). ZNF185 is located on chromosome Xq28, a chromosomal region of interest as a result of the more than 20 hereditary diseases mapped to this region. The ZNF185 LIM is a cysteine-rich motif that coordinately binds two zinc atoms and mediates protein-protein interactions. Heiss et al (29) cloned a full-length ZNF185 cDNA and showed that the transcript is expressed in a very limited number of human tissues with most abundant expression in the prostate.

Significantly, the present invention is the first identification of a correlation of ZNF185 regulation and cancer. Specifically, there was a significant down-regulation in the expression of ZNF185 gene in all prostate cancer tissues compared to benign prostatic tissues (Fig. 1 and 2b). The decrease in ZNF185 expression in prostate tumors indicated that ZNF185 plays an important role in the development and progression of prostate cancer.

To study the transcriptional silencing of ZNF185 in prostate cancer, LAPC4, LNCaP and PC3 prostate cancer cell lines were treated with 5-Aza-CdR an inhibitor of DNA methyl transferase DNMT1 (34). Treatment with 5-Aza-CdR showed approximately a 2.0-fold increase in mRNA levels of ZNF185 (Fig 3a, indicating that the gene might be partially silenced by methylation. To confirm the transcriptional inactivation, MSP was carried out to assess the methylation status of cytosine residues in the 5' CpG dinucleotides of genomic DNA in prostate tumors, adjacent benign tissues and in prostate cell lines with or without treatment with 5-Aza-CdR. Cytosine methylations within CpG dinucleotides were observed in the prostate cancer tissues and cell lines with two sets of primers used for PCR (Fig 3c). A reduction of the methylated band and increase of the unmethylated band in cell lines with 5-Aza-CdR treatment is consistent with the restoration of ZNF185 mRNA levels after demethylation. (Fig 3a).

In most of tissues samples, DNA not treated with bisulfite (unmodified) failed to amplify with either set of methylated or unmethylated specific primers but readily amplified with primers specific for the sequence before modification, suggesting an almost complete bisulfite reaction. Methylation of ZNF185 was accompanied by amplification of the unmethylated reaction as well. The presence of the unmethylated ZNF185 DNA could indicate the presence of normal tissues in

these non-microdissected samples. However, heterogeneity in the patterns of methylation in the tumor itself might also be present. Fisher's unordered test for methylation difference in metastatic, confined tumors and benign tissues was highly significant ($p<0.0003$).

The incidence of methylation in cancer tissues is shown in Fig. 3(d). Methylation status and down-regulation in the mRNA expression is correlated with higher tumor grade and metastasis.

These results indicate that methylation of CpG dinucleotides may be the major factor causing transcriptional inactivation of ZNF185 and repressing its expression in the prostate cancer tissues.

In summary, mRNA expression analysis with oligonucleotide microarrays identified a set of genes that characterize prostate cancer and benign prostatic tissues. A decrease in the expression of genes PSP94, BPAG1 and ZNF185 highly correlates with prostate cancer progression. Increase of Erg-2 levels also indicates its role in development of prostate cancer.

Significantly, this is the first study to identify inactivation of the LIM domain gene ZNF185 in patients with prostate cancer and in prostate cancer cell lines. The present invention identifies this gene as a marker of prostate cancer aggressiveness. According to the present invention, transcriptional silencing of PSP94 and BPAG1 additionally serves as prognostic markers for prostate cancer progression, and as potential therapeutic targets for prostate cancer.

TABLE 1. Prostate tissue samples with preoperative PSA values at diagnosis, Gleason histological scores, and metastasis status of the tissues.

Gleason grade/Lymph node	Sample ID	Age	Preop PSA (ng/ml)	TNM (97)	Metastatic site
6/Negative	6N 1	55	9.4	T2b,N0-	
	6N 2	50	7.5	T2b,N0-	
	6N 3	57	10.3	T2b,N0-	
	6N 4	67	16.7	T2b,N0-	
	6N 5	68	8.1	T2a,N0-	
6/Positive	6P 1	71	17.1	T2b,N1+	
	6P 2	61	5.2	T2b,N0+	
	6P 3	71	41.0	T2b,N0+	
	6P 4	65	7.0	T2a,N0+	
	6P 5	51	14.3	T2b,N0+	
	6P 6	66	23.5	T2b,N0+	
9/Negative	9N 1	67	21.6	T3a,N0-	
	9N 2	65	29.4	T3b,N0-	

Gleason grade/Lymph node	Sample ID	Age	Preop PSA (ng/ml)	TNM (97)	Metastatic site
	9N 3	65	24.9	T3b,N0-	
	9N 4	54	50.0	T3b,N0-	
	9N 5	59	25.8	T3b,N0-	
	9N 6	71	6.1	T3b,N0-	
9/Positive	9P 1	66	4.5	T3a,N0+	
	9P 2	65	6.69	T3b,N0+	
	9P 3	76	7.6	T3b,N1+	
	9P 4	71	467.0	T3b,N0+	
	9P 5	69	5.6	T3b,N0+	
	9P 6	66	2.9	T3b,N1-	
Metastatic	Met 1	62	0.15		Liver
	Met 2	72	97.3		Peritoneum
	Met 3	49	0.15		Lymph node
	Met 4	60	18.4		Lymph node
	Met 5	68	8.9		Lung

Literature Cited

1. DeMarzo, A.M., Nelson, W.G., Isaacs, W.B., Epstein, J.I. Pathological and molecular aspects of prostate cancer. Lancet, *361*: 955-964, 2003.

2. Dhanasekaran, S.M., Barrette, T.R., Ghosh, D., Shah, R., Varambally, S., Kurachi, K., Pienta, K.J., Rubin, M.A., Chinnaiyan, A.M. Delineation of prognostic biomarkers in prostate cancer. Nature, *412:* 822-826, 2001.

3. Luo, J., Duggan, D.J., Chen, Y., Sauvageot, J., Ewing, C.M., Bittner, M.L., Trent, J.M., Isaacs, W.B. Human prostate cancer and benign prostatic hyperplasia: Molecular dissection by gene expression profiling. Cancer Res., *61:* 4683-4688, 2001.

4. Magee, J.A., Araki, T., Patil, S., Ehrig, T., True, L., Humphrey, P.A., Catalona, W.J., Watson. M.A., Milbrandt, J. Expression profiling reveals hepsin overexpression in prostate cancer. Cancer Res., *61:* 5692-5696, 2001.

5. Welsh, J.B., Sapinoso, L.M., Su, A.I., Kern, S.G., Wang-Rodriguez, J., Moskaluk, C.A., Frierson, H.F. Jr., Hampton, G.M. Analysis of gene expression identifies candidate markers and pharmacological targets in prostate cancer. Cancer Res., *61:* 5974-5978, 2001.

6. Rubin, M.A., Zhou, M., Dhanasekaran, S.M., Varambally, S., Barrette, T.R., Sanda, M.G., Pienta, K.J., Ghiesh, D., Chinnaiyan, A.M. α-methyl coenzyme A racemase as a tissue biomarker for prostate cancer. JAMA, *287:* 1662-1670, 2002.

7. Ernst, T., Hergenhahn, M., Kenzelmann, M., Cohen, C.D., Bonrouhi, M., Weninger, A., Klaren, R., Grone, E., Wiesel, M., Gudemann, C., Kuster, J., Schott, W., Staehler, G., Kretzler, M., Hollstein, M., and Grone, H.J. Decrease and gain of gene expression are equally discriminatory markers for prostate carcinoma: a gene expression analysis on total and microdissected prostate tissue. Am. J. Pathol., *160:* 2169-2180, 2002.

8. Ehrlich, M. DNA methylation in cancer: too much, but also too little. Oncogene, *21:* 5400-5413, 2002.

9. Wong, I.H.N., Lo, Y.M.D., Zhang, J. Detection of aberrant p16 methylation in the plasma and serum of liver cancer patients. Cancer Res., *59:* 71-73, 1999.

10. Jones, P.A., and Baylin, S.B. The fundamental role of epigenetic events in cancer. Nat. Rev. Genet., *3:* 415-428, 2002.

11. Plass, C. Cancer epigenomics. Hum. Mol. Genet., *11:* 2479-2488, 2002.

12. Wong, I.H.N., Lo, Y.M.D. New markers for cancer detection. Cancer Prev., *4:* 471-477, 2002.

13. Giordano, T.J., Shedden, K.A., Schwartz, D.R., Kuick, R., Taylor, J.M., Lee, N., Misek, D.E., Greenson, J.K., Kardia, S.L., Beer, D.G., Rennert, G., Cho, K.R., Gruber, S.B., Fearon, E.R., and Hanash, S. Organ-specific molecular classification of primary lung, and ovarian adenocarcinomas using gene expression profiles. Am. J. Pathol., *159:* 1231-1238, 2001.

14. Herman, J.G., Graff, J.R., Myohanen, S., Nelkin, B.D., and Baylin, S.B. Methylation-specific PCR: A novel PCR assay for methylation status of CpG islands. Proc. Natl. Acad. Sci. USA, *93:* 9821-9826, 1996.

15. Esteller, M., Corn, P.G., Baylin, S.B., and Herman, J.G. A gene hypermethylation profile of human cancer. Cancer Res., *61:* 3225-3229, 2001.

16. Eisen, M.B., Spellman, P.T., Brown, P.O., and Botstein, D. Cluster analysis and display of genome-wide expression patterns. Pro. Natl. Acad. Sci. USA, *95:* 14863-14868, 1998.

17. LaTulippe, E., Satagopan, J., Smith, A., Scher, H., Scardino, P., Reuter, V., Gerald, W.L. Comprehensive gene expression analysis of prostate cancer reveals distinct transcriptional programs associated with metastatic disease. Cancer Res., *62*: 4499-506, 2002.

18. Luo, J.H., Yu, Y.P., Cieply, K., Lin, F., Deflavia, P., Dhir, R., Finkelstein, S., Michalopoulos, G., Becich, M. Gene expression analysis of prostate cancers. Mol Carcinog., *33*: 25-35, 2002.

19. Shields, J.M., Rogers-Graham, K., Der, C.J. Loss of transgelin in breast and colon tumors and in RIE-1 cells by Ras deregulation of gene expression through Raf-independent pathways. J. Biol. Chem., *277:* 9790-9799, 2002.

20. Rhodes, D.R., Barrette, T.R., Rubin, M.A., Ghosh, D., Chinnaiyan, A.M. Meta-analysis of microarrays: interstudy validation of gene expression profiles reveals pathway dysregulation in prostate cancer. Cancer Res., *62*: 4427-4433, 2002.

21. Stamey, T.A., Warrington, J.A., Caldwell, M.C., Chen, Z., Fan, Z., Mahadevappa, M., McNeal, J.E., Nolley, R., Zhang, Z. Molecular genetic profiling of Gleason grade 4/5 prostate cancers compared to benign prostatic hyperplasia. J. Urol., *166*: 2171-2177, 2001.

22. Sakai, H., Tsurusaki, T., Kanda, S., Koji, T., Xuan, J.W., and Saito, Y. Prognostic significance of β-microseminoprotein mRNA expression in prostate cancer. Prostate, *38:* 278-284, 1999.

23. Garde, S.V., Basrur, V.S., Finkelman, M.A., Krishnan, A., Wellham, L., Ben-Josef, E., Garde, H. S.V., Basrur, V.S., Finkelman, M.A., Krishnan, A., Wellham, L., Ben-Josef, E., Haddad, M., Taylor, J.D., Porter, A.T., Tang, D.G. Prostate secretory protein (PSP94) suppresses the growth of androgen-independent prostate cancer cell line (PC3) and xenografts by inducing apoptosis. Prostate, *38:* 118-125, 1999.

24. Bergstraesser, L.M., Srinivasan, G., Jones, J.C., Stahl, S., Weitzman, S.A. Expression of hemidesmosomes and component proteins is lost by invasive breast cancer cells. Am. J. Pathol., *147*: 1823-1839, 1995.

25. Herold-Mende, C., Kartenbeck, J., Tomakidi, P., Bosch, F.X. Metastatic growth of squamous cell carcinomas is correlated with upregulation and redistribution of hemidesmosomal components. Cell Tissue Res., *306:* 399-408, 2001.

26. Simpson, S., Woodworth, C.D., DiPaolo, J.A. Altered expression of Erg and Ets-2 transcription factors is associated with genetic changes at 21q22.2-22.3 in immortal and cervical carcinoma cell lines. Oncogene, *14*: 2149-2157, 1997.

27. Antonyak, M.A., McNeill, C.J., Wakshlag, J.J., Boehm, J.E., Cerione, R.A. Activation of the Ras-ERK Pathway Inhibits Retinoic Acid-induced Stimulation of Tissue Transglutaminase Expression in NIH3T3 Cells. J. Biol. Chem., *278*: 15859-15866, 2003.

28. Lozano, E., Betson, M., Braga, V.M. Tumor progression: Small GTPases and loss of cell-cell adhesion. Bioessays, *25*: 452-463, 2003.

29. Heiss, N.S., Gloeckner, G., Bachner, D., Kioschis, P., Klauck, S.M., Hinzmann, B., Rosenthal, A., Herman, G.E., Poustka, A. Genomic structure of a novel LIM domain gene (ZNF185) in Xq28 and comparisons with the orthologous murine transcripts. Genomics, *43:* 329-338, 1997.

30. Bach, I. The LIM domain: regulation by association. Mech Dev *91*: 5-17, 2000.

31. McLoughlin, P., Ehler, E., Carlile, G., Licht, J.D., Schafer, B.W. The LIM-only protein DRAL/FHL2 interacts with and is a corepressor for the promyelocytic leukemia zinc finger protein. J. Biol. Chem., *277:* 37045-37053, 2002.

32. Mousses, S., Bubendorf, L., Wagner, U., Hostetter, G., Kononen, J., Cornelison, R., Goldberger, N., Elkahloun, A.G., Willi, N., Koivisto, P., Ferhle, W., Raffeld, M., Sauter, G., and Kallioniemi, O.P. Clinical validation of candidate genes associated with prostate cancer progression in the CWR22 model system using tissue microarrays. Cancer Res., *62:* 1256-1260, 2002.

33. Yamada, Y., Pannell, R., Forster, A., and Rabbitts, T.H. The LIM-domain protein Lmo2 is a key regulator of tumor angiogenesis: a new anti-angiogenesis drug target. Oncogene, *21:* 1309-1315, 2002.

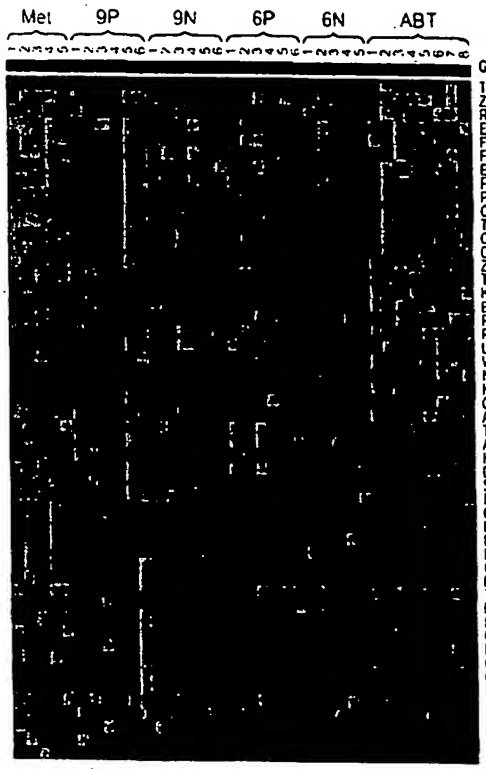
34. Robert, M.F., Morin, S., Beaulieu, N., Gauthier, F., Chute, I.C., Barsalou, A., and MacLeod, A.R. DNMT1 is required to maintain CpG methylation and aberrant gene silencing in human cancer cells. Nat. Genet., *33:* 61-65, 2003.

CLAIMS

We claim:

ABSTRACT

The present invention provides, *inter alia*, novel methods and compositions for the diagnosis, staging and prognosis of prostate cancer, based on transcriptional silencing of gene expression, including of zinc finger protein 185 (ZNF 185), prostate secretory protein (PSP94) and bullous pemphigoid antigen (BPAG), and based on DNA methylation.



Gene	UniGene	Expression ratio to mean ABT				
		Met	9P	9N	6P	6N
TGM4	Hs.2387	32.70+21.83	17.46+21.27	12.40+17.11	5.23+3.92	23.47+19.76
ZFP36	Hs.343586	4.68+3.55	1.98+1.34	2.03+1.94	1.86+1.53	3.95+1.83
RGS1	Hs.35661	4.07+4.35	2.28+3.19	2.85+3.95	2.39+6.51	1.51+2.05
EFS2	Hs.24587	7.37+1.91	3.68+0.75	2.03+0.36	1.82+0.44	2.61+0.79
FLH2	Hs.8302	3.18+1.26	1.79+1.20	1.82+1.72	2.58+2.71	2.38+2.51
FOXF1	Hs.155591	6.43+4.01	2.34+1.42	2.19+0.87	3.19+2.26	2.88+2.18
ENIGMA	Hs.102948	3.73+2.16	2.21+1.10	1.42+0.92	3.38+2.43	3.28+3.05
FHL1	Hs.239069	5.25+2.69	1.94+0.87	1.82+0.97	3.40+1.71	2.78+1.85
PCP4	Hs.80296	32.45+12.40	3.06+1.64	2.36+1.51	5.22+4.09	5.38+2.14
CNN1	Hs.21223	52.53+27.61	4.59+1.95	3.12+1.38	5.17+3.69	4.09+2.72
TAGLN	Hs.75777	9.07+3.52	2.46+0.68	1.46+0.64	2.56+1.58	2.55+1.87
GSTM1	Hs.301961	4.10+0.87	3.58+1.83	2.07+0.69	2.92+0.91	3.39+2.85
CSRP1	Hs.198680	7.89+3.31	3.85+1.80	2.75+0.90	3.15+1.28	3.46+1.89
ZNF185	Hs.16622	11.17+4.38	3.21+1.30	3.89+0.82	3.67+1.61	2.63+0.56
TRIM29	Hs.82237	6.26+2.82	3.53+1.98	2.79+2.36	3.84+3.21	2.87+1.95
KRT5	Hs.195650	63.86+80.24	6.67+3.12	5.24+2.81	4.76+2.44	4.76+4.10
BPAG1	Hs.198699	14.04+6.03	3.75+1.74	5.03+4.19	4.14+2.10	7.76+5.92
PLP1	Hs.1787	5.09+3.00	2.54+1.74	2.08+2.45	3.91+2.75	3.27+2.53
PSP94	Hs.183752	129.56+59.19	3.18+2.12	4.14+1.34	2.36+1.61	2.68+1.82
LOC113146	Hs.57548	9.20+7.48	3.27+1.41	1.14+0.73	4.34+1.48	3.29+1.65
WFDC2	Hs.2719	11.21+3.80	2.71+0.62	4.35+2.78	5.24+2.49	3.14+2.64
NEFH	Hs.198760	14.89+7.11	17.22+14.17	4.59+1.85	2.85+1.74	1.69+0.75
KRT15	Hs.80342	90.71+213.95	6.38+5.01	5.24+5.63	4.68+5.26	4.45+4.92
GAGEC1	Hs.95420	63.35+27.88	2.58+0.84	2.39+0.69	1.59+0.46	2.58+0.85
ACPP	Hs.1852	7.22+2.24	2.23+1.07	3.87+3.07	1.00+1.90	1.24+2.09
TMSNB	Hs.56145	3.13+7.91	2.97+5.26	2.55+5.63	4.64+8.58	3.85+2.95
AMACR	Hs.128749	5.70+12.34	3.66+9.49	1.72+2.22	0.65+0.32	1.01+0.57
HPN	Hs.823	2.99+3.64	3.19+7.31	2.80+2.96	3.93+2.46	4.19+1.96
DKFZP564B167	Hs.76285	2.50+3.43	2.22+5.19	1.62+1.09	3.04+2.72	2.46+2.17
STEAP	Hs.61635	2.35+6.70	2.65+4.94	2.37+2.59	2.64+1.49	1.88+0.78
FOLH1	Hs.1915	3.76+10.12	2.61+3.17	1.90+2.11	2.14+1.89	3.01+2.15
CADPS	Hs.151301	10.26+11.52	2.30+2.56	1.04+0.58	0.25+0.19	1.13+0.40
LOC90355	Hs.25925	3.87+2.24	2.87+2.07	0.77+1.18	1.91+2.13	2.21+1.99
ERG2	Hs.45514	20.06+27.86	8.36+9.15	4.88+5.29	5.06+7.52	19.69+12.87
MACMARCKS	Hs.75061	3.08+12.35	2.62+4.51	2.45+7.68	2.95+4.70	4.02+2.80
GPC3	Hs.119651	4.79+1.19	5.73+8.89	1.79+1.88	2.01+2.39	1.34+1.20
KIAA0101	Hs.81892	5.51+5.53	5.43+5.69	2.08+0.51	2.76+0.95	2.90+0.44
TK1	Hs.105097	6.61+4.25	3.55+2.14	2.45+1.30	2.18+1.59	1.95+1.06
MAD2L1	Hs.79078	3.44+10.86	4.18+12.06	5.26+6.36	4.85+5.57	4.73+7.78
KIAA0906	Hs.56966	4.60+13.94	4.13+6.82	2.21+3.95	3.19+4.26	5.33+4.46
CCNB2	Hs.194698	5.94+7.85	4.05+11.25	2.10+2.52	2.47+3.83	2.83+2.65
BUB1B	Hs.103834	2.37+5.29	2.99+5.24	2.13+1.64	1.60+2.19	2.08+1.36
CCNB1	Hs.23960	4.31+2.88	3.07+6.25	2.12+2.07	2.23+0.82	2.13+1.05
DEEPEST	Hs.16244	5.12+1.55	5.71+7.67	2.23+1.37	1.62+1.52	2.11+1.49
KIAA0186	Hs.36232	5.32+3.32	2.91+2.60	1.43+0.52	2.42+1.13	1.16+0.98
TNRC9	Hs.110826	6.76+6.11	8.22+6.60	2.16+1.14	6.09+2.54	8.03+2.95
RAP2A	Hs.355373	3.87+9.42	1.24+1.51	0.81+0.68	0.93+1.21	1.44+0.67
F2R	Hs.128087	5.65+11.11	10.49+13.61	8.82+13.08	17.51+15.26	21.01+15.34
ICAP-1alpha	Hs.356320	27.64+88.62	5.57+25.47	1.55+2.45	0.96+0.46	0.93+0.65
ARHGDIB	Hs.83656	4.04+1.27	3.02+3.17	1.59+0.79	2.09+0.69	2.19+0.53

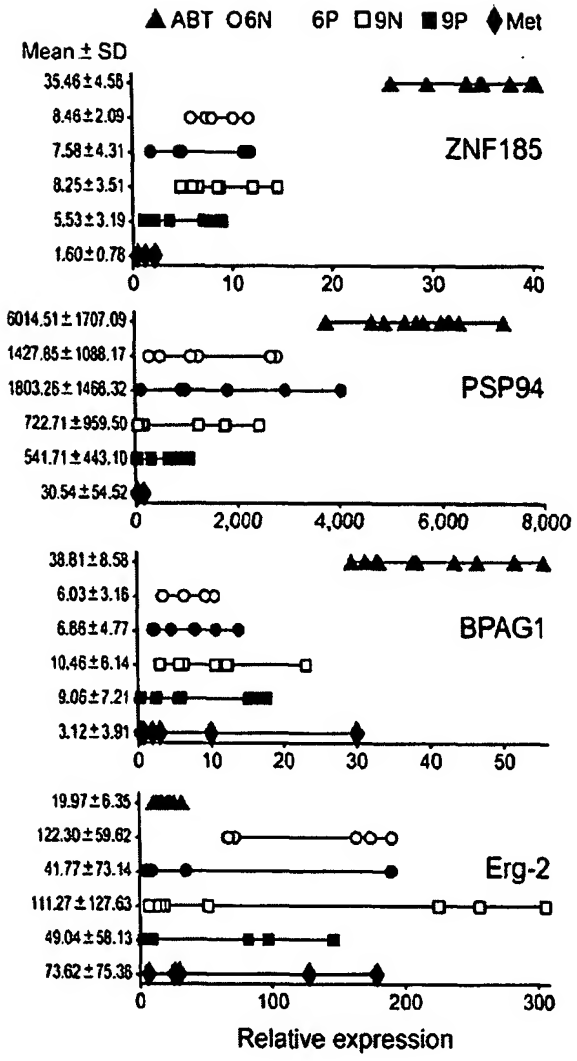
Fold decrease

Fold increase

Fig. 1

Gene		Primers and Probe	Amplicon bp
ZNF185	FP	TGG ATG AAA GGC AAG GTA AAG AG	84
	RP	TTC TAA AAC TCC CTT AAA GGC AGA CT	
	Probe	CCA AGA TAG GCT GGC TTC CCC CG	
PSP94	FP	AGT GAA TGG ATA ATC TAG TGT GCT TCT AGT	100
	RP	GCA TGG CTA CAC AAT CAT TGA CTA T	
	Probe	CCC AGG CCA GGC CTC ATT CTC CT	
BPAG1	FP	TCG CTG AAA GAG CAC GTC AT	94
	RP	AGC AAT CTA AAA CAC TGC AGC TTG	
	Probe	AAT CAA AGA GAA AGA TAT AAA TTC GTT CCC ACA GCC	
Erg-2	FP	TCC TGT CGG ACA GCT CCA AC	75
	RP	CGG GAT CCG TCA TCT TGA	
	Probe	TGC ATC ACC TGG GAA GGC ACC AAC	

Fig. 2A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Mean ± SD
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8.46±2.09
7.58±4.31
8.25±3.51
5.53±3.19
1.60±0.78
ZNF185
0 10 20 30 40
6014.51±1707.09
1427.85±1088.17
1803.26±1466.32
722.71±959.50
541.71±443.10
30.54±54.52
PSP94
0 2,000 4,000 6,000 8,000
38.81±8.58
6.03±3.16
6.86±4.77
10.46±6.14
9.05±7.21
3.12±3.91
BPAG1
0 10 20 30 40 50
19.97±6.35
122.30±59.62
41.77±73.14
111.27±127.63
49.04±58.13
73.62±75.38
Erg-2
0 100 200 300
Relative expression

Fig. 2B

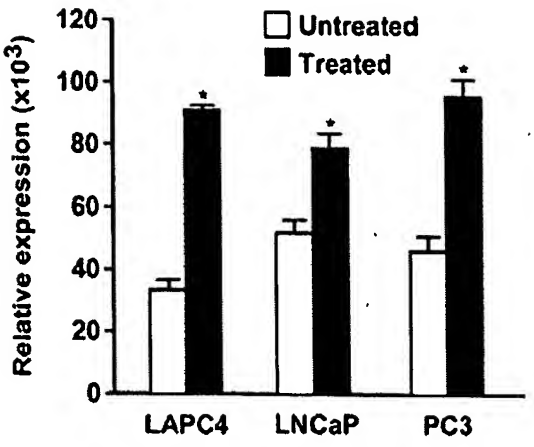
Untreated
Treated
Relative expression (x10^3)
120
100
80
60
40
20
0
LAPC4
LNCaP
PC3

Fig. 3A

Primer set			Size (bp)	Genomic position
1W	FP	GCGCAGTTCCGGGTGTCTGTC	197	210
	RP	GCGGGGAGGACCAGCGTTAG		
1M	FP	GCGTAGTTTCGGGTGTTTG	197	210
	RP	ACGAAAAAAACCAACGTTAACTA		
1U	FP	GIGTAGTTTIGGGTGTTTGTTAGG	196	210
	RP	CAAAAAAAACCAACATTAACTATTCTC		
2W	FP	CCTGGGACTCCGTCAGACTGG	146	335
	RP	GACAGACACCCGGAACTGCG		
2M	FP	TTGGGATTTCGTTAGATTGG	145	335
	RP	AACAAACACCCGAAACTACG		
2U	FP	TGGGATTTTIGTTAGATTGGAAAGG	146	333
	RP	CTAACAAACACCCAAAACTACACCA		

Fig. 3B

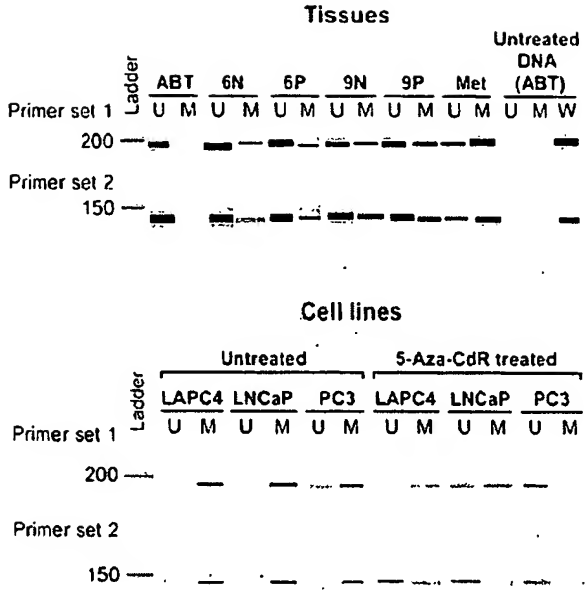
Tissues
Ladder
ABT
6N
6P
9N
9P
Met
Untreated DNA (ABT)
U M U M U M U M U M U M U M W
Primer set 1
200
Primer set 2
150
Cell lines
Untreated
5-Aza-CdR treated
Ladder
LAPC4
LNCaP
PC3
LAPC4
LNCaP
PC3
U M U M U M U M U M U M
Primer set 1
200
Primer set 2
150

Fig. 3C

Samples	Total	Methylated (%)
Benign prostatic tissues	12	0 (0)
Gleason Score 6 tumors	11	4 (36.3)
Gleason Score 9 tumors	14	7 (50)
Metastatic tissues	5	5 (100)

Fig. 3D

SEQUENCE LISTING

<110> Vanaja, Donkena Krishna
Young, Charles Y.F.

<120> Methods And Compositions For Diagnosis, Staging and Prognosis of Prostate Cancer

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Glu Glu Glu Glu Val Val Pro Phe Ser Ser Asp Glu Gln Lys Arg Arg
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tca gag gct gca agc ggt gtt ctg agg agg aca gct ccc cgg gag cac      199
Ser Glu Ala Ala Ser Gly Val Leu Arg Arg Thr Ala Pro Arg Glu His
    40                  45                  50

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Ser Tyr Val Leu Ser Ala Ala Lys Lys Ser Thr Gly Ser Pro Thr Gln
55                  60                  65

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Glu Thr Gln Ala Pro Phe Ile Ala Lys Arg Val Glu Val Val Glu Glu
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Thr Pro Gly Ser Asn Ser Ser Arg Gly Glu Glu Ile Val Arg Leu Gln
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Lys Ser Asn Ser Thr Ala Ala Gln Glu Asp Ala Lys Ala Asp Pro Lys
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Gly Ala Leu Ala Asp Cys Glu Gly Lys Asp Val Pro Thr Arg Val Gly
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Glu Ala Trp Gln Glu Arg Pro Gly Ala Pro Arg Gly Gly Gln Gly Asp
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Gln Ser Ser Pro Ser Gly Ser Glu Gln Leu Val Arg Arg Glu Ser Cys
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Val Gly Glu Ala Trp Gln Asp Arg Pro Arg Ala Pro Arg Gly Gly Gln
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Gly Asp Pro Ala Val Pro Thr Gln Gln Pro Ala Asp Pro Ser Thr Pro
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